Irish Journal of Agricultural and Food Research **50:** 31–46, 2011

Ryegrass breeding – balancing trait priorities

A. Stewart[1,†] and R. Hayes[2]

[1]*PGG Wrightson Seeds, P.o. Box 175, Lincoln, New Zealand 7640*

[2]*IBERS, Aberystwyth University, Aberystwyth, Ceredigion, SY23 3EB, UK*

In all ryegrass breeding programmes it is necessary to select a range of traits within different cultivar types, varying in ploidy and flowering time. The traits selected in ryegrass breeding can be broadly grouped into production traits such as yield, quality and persistency; those seed production traits crucial for delivery of the cultivar, as well as those traits that can benefit the environment, or allow ryegrass to be used for biofuel production. The emphasis placed on each trait will depend on its economic value within the various farming systems where each cultivar will ultimately be used, as well as the potential to make genetic gain in each trait. In all cases multiple trait selection will be required, to develop a cultivar improved for key traits of interest but importantly the cultivar must not have unacceptable performance for any trait. Where the genetic variation is inadequate within perennial ryegrass it may be necessary to enhance ryegrass diversity. In the future this could be achieved through targeted introgression from the closely related Festuca species, or through introduction of genes via genetic modification. Funding of ryegrass breeding internationally will increasingly be subject to the economic success of a few larger seed companies as Government funding for field-based breeding is diminishing and shifting focus to more basic research, often of a molecular nature. Ensuring this expensive basic research and associated molecular technologies are used effectively in ryegrass breeding programmes will remain a challenge when seed companies operating field-based programmes are vulnerable to considerable economic pressure.

Keywords: breeding; perennial ryegrass; traits

†Corresponding author: astewart@pggwrightsonseeds.co.nz

Introduction

The question of prioritising traits under selection in ryegrass breeding programmes is not always simple, because each cultivar must have excellent performance for a multiplicity of factors, and importantly must not have unacceptable performance in any. A cultivar may be used in different regions and different farming systems, as well as in pure or mixed swards with legumes. In the various farming systems there are also roles for different ryegrass species and types, from perennial ryegrass, hybrid ryegrass, Italian ryegrass (biennial) through to westerwolds ryegrass (annual), each varying in maturity date, ploidy level, seasonal growth pattern, forage quality and persistency.

The forage breeder's goal is to develop cultivars that will improve animal performance on farms. However, perennial cultivars need be able to persist under the local climatic extremes, cope with pests and diseases, as well as having adequate seed yield for competitive commercial delivery. The breeder must also work within the prevailing regulatory environment, as well as the changing economic and funding conditions.

Access to suitable germplasm or genetic variation for the trait of interest is crucial and suitable variation is not always available within the species. Although genetic modification allows us to transfer genes from any other life form, this is not a viable option in many countries for regulatory, funding or public perception reasons.

In countries where cultivars may not be sold without first going through a compulsory Recommended or National List evaluation system, the traits that a breeder needs to select for are strongly dictated by the testing system. In addition the authorities may not measure, or not have the resources to measure, more complex traits which could be important in some on-farm situations. This is particularly true for persistency under grazing where animals are not utilised within these mandatory evaluation systems. They may also only carry out trials to simulate certain farm management systems and ignore other systems.

The desirable traits selected for in ryegrass breeding programmes can be broadly divided into those that influence production: forage yield, forage quality and persistency, those that affect seed production traits crucial for delivery of the cultivar, and those that influence the environment. The majority of production traits have been core breeding targets since modern ryegrass breeding began, but in recent years breeding to minimise environmental impacts has become very important, as agricultural practices have intensified and their consequences have become understood.

Production traits

Forage yield

One of the major objectives of all forage breeding programmes is improving forage yield. This is not as simple for pasture as for single harvest grain crops, where harvest index can be improved. Pasture harvest involves the near total removal of above ground biomass with consequent loss of photosynthetic capacity, and there is a demand for a continuous supply of fodder throughout the year. Small increases in winter or early spring production may be worth disproportionately more than total spring production. McEvoy, O'Donovan and Shalloo (2010) have reported that winter yield is worth up to 5 times the value of spring and summer yield in Irish dairy systems. For this reason breeding for an appropriate seasonal yield must take precedence over total annual yield, and testing systems need to reflect this.

In New Zealand, winter growth has always been valuable as temperatures are milder than in the UK and Ireland. Such winter activity has been achieved in many pasture species in New Zealand by the incorporation of Mediterranean germplasm (Stewart 2006). Care must be taken with breeding for winter growth in regions where cold winters occur, and a balance must be struck between the level of winter growth and winter hardiness. It is possible, though, to combine strong early spring growth with a suitable level of winter hardiness even for colder winter regions.

The time of heading of a cultivar can have a large effect on the timing of early spring growth and overall yield. Any benefits in seasonal and total yield due to early flowering must be balanced against the decline of mid-season quality and poorer persistency frequently observed with early cultivars in Ireland (Brereton and McGilloway 1999). It is likely that with some breeding effort these deficiencies can be overcome.

The general trend in climate warming may allow more winter active cultivars to be used in the European region than in the past, but care will be required that winter hardiness is maintained for the occasional very cold winter. Although recently the possibility has been suggested that Europe will experience more frequent, severe winters as either the Gulf Stream continues to weaken (Seager *et al*. 2002; Minobe *et al*. 2008) or the effects of Jet Stream blocking intensify (Woollings 2010).

The genetic gain in annual dry matter (DM) yield of perennial ryegrass has been estimated from cutting trials in Europe and New Zealand as around 4 to 5% per decade, but less than 1% in the USA, where there is little perennial ryegrass breeding activity. Gains in seasonal yield may differ considerably reflecting the different breeding priorities. When the perennial nature of pasture is considered these genetic gains compare favourably with those achieved on major crops where there are more resources and a simple single-harvest (Woodfield 1999; Easton *et al*. 2002; Wilkins and Humphreys 2003; van der Heijden and Roulund 2010).

Forage quality

The ability of a cultivar to influence animal performance is not only related to feed quantity, but also to the metabolizable energy available to the animal, and any factor that can increase animal intake. This is often very complex, reflecting the dynamics of rumen digestion of cell wall components and cellular contents, and any factors that can reduce feed transit time through the rumen. Feed quality also varies enormously with flowering behaviour, clover content, leafiness, diseases, growth rate and many other management related factors. Although the genetic variation of many quality factors is small compared to the environmental component, any advance in quality of a cultivar is valuable.

Metabolizable energy is prohibitively expensive to measure directly on cultivars so more simple approximations are commonly determined on herbage in most pasture research. These usually involve data where previous experiments have been used to calibrate digestibility, neutral detergent fibre, acid detergent fibre, protein, starch, lipid, water soluble carbohydrates (WSC), fatty acid profile and many other quality related factors, often using near infrared spectroscopy.

One of the primary determinants of digestibility in all grasses relates to the decline in quality as flowering progresses. The timing of heading influences both the timing of this decline in quality and the timing of the spring growth flush. Effective use of a range of grasses with differing heading dates enables farmers to set up farming systems where seasonal feed availability more effectively meets animal demands. Often this will involve the additional use of brassica crops to both fill feed gaps and to enable the economic renewal of pasture.

Heading date also needs to be compatible with achieving an economic seed yield in the seed production region. For example, in New Zealand it is not possible to produce competitive seed yields from very late heading European perennial grasses.

The quality of ryegrass pastures once the flowering period is over – often termed mid-season quality – can be extremely important in many farming systems. This can be influenced by aftermath flowering in summer, and breeders normally select for minimal aftermath flowering behaviour to maximise digestibility. Reduced aftermath flowering can also enhance persistency, as cultivars with a high proportion of reproductive tillers are often vulnerable to poor recovery from grazing (Fulkerson and Donaghy 2001), but can also reduce the ability to reseed which may be important in some extensive farm systems.

Even in cultivars with no aftermath seed heads, the forage quality in mid-season can vary considerably, usually due to the ratio of plant parts, particularly leaf to psuedostem ratio, tiller size and other factors. In Ireland, late flowering cultivars generally have a higher proportion of green leaf and less pseudostem in the grazing horizon than intermediate flowering cultivars, resulting in a higher digestibility, greater DM intake and milk production (O'Donovan and Delaby 2005).

In pure ryegrass systems, where mid-season quality is a driver of production, this trait should be selected by breeders and progress evaluated in the cultivar testing systems. However, in mixed ryegrass and clover systems the clover content may potentially offer a larger improvement in quality than the small quality differences among cultivars.

The ploidy level of cultivars also influences forage quality, with tetraploids having larger cells and a higher ratio of cell content to cell wall resulting in a lower DM concentration. Typically, animals must consume more fresh weight of tetraploid cultivars to obtain the same intake of dry matter as from diploids. In pastures of similar digestibility, tetraploid perennial ryegrass cultivars have often, but not always, been shown to increase feed intake by 3 to 5%, with at least a similar improvement in animal production (van Bogaert 1975; Connolly, Riberio and Crowley 1977; Hageman *et al*. 1993; Vipond, Swift and McClelland 1993).

The value of these forage quality factors in tetraploids must be balanced against forage yield, as most of the tetraploid cultivars available today in the UK offer little advantage over diploids because they lack the yield and mid-season quality of the leading diploid cultivars (NIAB Recommended List 2009). Similarly, studies have shown that cattle may require more supplemental concentrates when reared for beef or milk production on current tetraploids rather than leading diploids (O'Donovan and Delaby 2005; Orr *et al*. 2005). However, in other countries, such as New Zealand, the leading tetraploid cultivars have competitive yields when compared to the leading diploids.

Breeding a ryegrass that allows more clover in the sward would be important for increased forage quality but little ryegrass breeding has been done for this characteristic. It is known, however, that some grasses, such as timothy and tall fescue, allow a greater proportion of white clover than ryegrass, and that this contributes to an increase in milk production (Thomson and Kay 2005). If this were due to less mid-spring suppression of clover, then breeding a ryegrass to maximise clover proportion may be possible, but it is clearly more complex than the challenge facing clover breeders who regularly breed and test their clover germplasm in ryegrass swards and select strongly for clover proportion (Evans, Williams and Evans 1996; Woodfield 1999; Abberton and Marshall 2010; Annicchiarico and Proietti 2010).

One of the few forage breeding programmes in the world to sustain prolonged focus on a single forage quality trait has been at IBERS in the UK. That programme has been focussed on fructan accumulation to increase WSC concentration in ryegrass, and this has resulted in a commercial range of "high sugar" grasses such as AberDart, AberMagic and AberGreen (Wilkins 1998; Wilkins and Lovatt 2007; Wilkins *et al*. 2010). Similarly, in New Zealand, the cultivar Expo has been developed with high WSC concentration (Easton *et al*. 2009; Rasmussen *et al.* 2009; Stewart *et al*. 2009).

Water soluble carbohydrates consist of simple sugars and longer chain fructans which act as major storage carbohydrates in grasses. Their levels depend on a wide range of factors, including the plant part, ploidy level, plant maturity, diurnal and seasonal effects, temperature, light intensity, growth rate, endophyte, water status, as well as the inherent differences between cultivars. Application of N fertiliser allows more rapid growth, and thus tends to result in lower levels of fructan accumulation. Fructan accumulation is greatest when swards are allowed to accumulate higher herbage mass, such as silage crops, with levels lower under very frequent grazing (Pollock and Cairns 1991; Rasmussen *et al*. 2008).

In New Zealand, where frequent grazing is commonly practiced, expression of WSC in cultivars bred for elevated WSC is frequently no different to normal types (Francis *et al*. 2006; Smith 2008; Allsop, Nicol and Edwards 2009) or only weakly expressed (Bryant *et al*. 2009; Cosgrove *et al*. 2010). It is expressed more under the longer cutting intervals of silage and biofuel crops, where it is valuable for silage fermentation and for biofuel extraction.

Animal grazing of pure swards with high versus low concentrations of WSC, have shown increases in animal performance over unselected cultivars in some trials but most trials have yielded non-significant animal performance advantages (Edwards, Parsons and Rasmussen 2007; Marley *et al*. 2007; Cosgrove *et al*. 2010; Parsons *et al*. 2010).

New selections with even higher levels of WSC continue to be developed, largely for biofuel purposes, as ruminants run the risk of acidosis in the rumen if levels are excessive and horses frequently succumb to laminitis when WSC concentrations are high (Longland and Byrd 2006). Further research will be required to determine the safe limits for WSC under a range of on-farm conditions for both horses and ruminants.

It is clear that there are many factors involved in improving the quality of ryegrass cultivars. It is likely that breeders will have to target multiple traits to ensure that cultivar improvements will consistently lift animal performance on farms.

Forage persistency

It is important that perennial cultivars persist in pastures and data from the UK show that, in general, modern cultivars have greater persistence than older ones (Camlin 1997). Many factors can be involved in lack of pasture persistency, pasture thinning and failure, only some of which may be genetic. Cultivars are known to vary in persistency and one of the factors that provides a ryegrass cultivar with more capacity to survive adverse conditions and better persistence is high tiller density (Camlin and Stewart 1978; Wilkins and Humphreys 2003), although this is often difficult for breeders to combine with high yield potential (Gilliland and Mann 2001).

Almost all germplasm used in breeding programmes today originates from plants from old persistent pastures, although many may have been subjected to a number of cycles of crossing and selection. Usually persistency is determined by the stability of DM yield of a cultivar over a number of years, and generally grasses that persist under frequent close cutting persist well under grazing (Wilkins and Humphreys 2003).

Cultivars must have adequate tolerance to the extreme stresses occurring on farms, such as winter cold stress, summer drought stress, intense defoliation, and treading damage. In Europe winter hardiness has long been crucial for persistency, while in New Zealand, summer drought tolerance is integrally associated with endophyte-mediated pest tolerance.

In situations where pests damage pasture it is necessary to breed cultivars resistant to, or tolerant of, these pests. This may include genetic resistances and/or endophyte-mediated resistance. In New Zealand, insect pest damage usually manifests itself during dry summer conditions when growth and tillering capacity are severely reduced. Under moist conditions, when ryegrass is actively growing, pest damage is usually less apparent, despite often being present. The pests involved include a number of both indigenous and introduced pests: porina (*Wiseana cervinata*), grass grub (*Costelytra zealandica*), mealy bug (*Balanococcus poae*), root aphid (*Aploneura lentisci*), Argentine stem weevil (*Listronotus bonariensis*), black beetle (*Heteronychus arator*), black field cricket (*Teleogryllus commodus*), Tasmanian grass grub (*Aphodius tasmaniae*), and grey field slug (*Deroceras reticulatum*).

The seed-borne endophytic fungus (*Neotyphodium lolii*), with which perennial ryegrass has co-evolved, produces a series of quite potent alkaloids that can provide substantial pest protection, significantly poorer animal production and, at times, ryegrass staggers (Easton 2007; Thom, Waugh and Minneé 2010). The influence of the endophyte on persistency varies depending on local conditions, but in pest prone environments of New Zealand endophyte-free plants may fail to survive one summer (Hume, Cooper and Panckhurst 2009; Popay and Thom 2009). Even in Ireland, where pest pressure is obviously much less, older pastures may contain a significant proportion of endophyte-infected plants, suggesting that it may provide a natural advantage, perhaps by protecting the plant from overgrazing by livestock, if not resistance to minor insect pests (Ribeiro *et al*. 1996).

The discovery and commercialisation of the “safer” endophyte strains, AR1 and AR37, have been a breakthrough for animal production and pest protection in New Zealand (Woodfield and Easton 2004). However, pastures with “safer” endophytes appear to be more vulnerable to overgrazing than were the unpalatable “wildtype” endophytic pastures (Rennie, King and Bell 2010).

Disease resistance

In general, perennial ryegrass has few major diseases that reduce forage yield, but resistance to crown rust (*Puccinia coronata*) and, in some regions, Drechslera leaf spot (*Drechslera siccans*), mildew and rhyncosporium leaf spot is useful (Connolly 2001). Similarly, resistance to diseases of seed production, such as stem rust (*Puccinia graminis*), is also important.

Breeding for disease resistance can often be simply done in the field by removing susceptible plants but, for some diseases, the grass population is not reliably and uniformly exposed to the disease, and glasshouse or laboratory screening techniques may be employed. In some programmes molecular markers are being investigated for crown rust resistance (Thorogood *et al*. 2001; Schejbel *et al*. 2007).

Barley yellow dwarf virus and ryegrass mosaic virus are known to be widespread in older pastures and these may have a significant impact on performance and persistence and some breeding programmes have involved selection against them (Wilkins 1974; Wilkins and Catherall 1977; Latch 1977; Webster *et al*. 1996).

Seed delivery traits

Economic delivery of seed to farmers is crucial. Breeders report many instances of cultivars that have failed to be delivered successfully to farmers despite offering excellent or even exceptional production. A New Zealand example was the cultivar Tolosa that had exceptional forage yield but very poor seed production.

The most important trait involved in effective delivery is undoubtedly seed yield and, in New Zealand, endophyte infection levels. Seed yield can be influenced by many factors, including heading date, shattering resistance and resistance to stem rust, response to fungicides, N fertiliser input and growth regulators (Elgersma 1990). In New Zealand, where novel endophytes are used, it is often necessary for the breeder to co-select ryegrass to enable high transmission of endophyte into seed (Easton 2007). Although seed crop management techniques have improved (Rolston, Chynoweth and Stewart 2006; Rolston *et al*. 2007) a cultivar still needs to have a competitive seed yielding ability. In the future, marker assisted selection may offer potential to improve seed yield within forage crops that are otherwise difficult to improve (Armstead *et al*. 2008).

Environmental and biofuel traits

Nitrogen in the environment is of concern, particularly in drinking water. In many farming regions part of the applied N fertiliser, N in animal urine and indeed from any source, including clovers, may be leached (Sprosen, Ledgard and Thom 1977). This occurs primarily during winter, when rainfall is often high and temperature limits plant uptake and growth (Stewart 2001). The introduction of the nitrate vulnerable zones directive by the EU has raised awareness among European farmers of the importance of N fixed by legumes and the use of crops which have been bred for improved N utilisation.

Breeding for N use efficiency and less leaching of N has become very important in the EU as regulations limit N fertiliser applications (Wilkins, Allen and Mytton 2000). Increasing WSC increases the WSC/protein ratio leading to an increase in the efficiency of N capture in the rumen, leaving less N in the urine and less potential for N leaching into the environment (Merry *et al*. 2006). Unfortunately, this mechanism is not very effective in heavily N-fertilised pastures, where it is most needed, but functions for low protein pastures where it is least needed (Edwards *et al*. 2007; Abberton *et al*. 2008; Morgavi *et al*. 2010). In these situations intercepting N before it is leached may be a better option by selecting for improvements in rooting depth (Crush and Nichols 2010).

Phosphorous in the environment is of concern and phosphatic rock supplies are limited. In contrast to the leaching losses of N, most losses of P are due to soil erosion, and soil management practices will ultimately be more significant than plant factors in preventing losses. Breeding for P use efficiency, though, could be of considerable value, more so for clover use than ryegrass. Unfortunately, breeding for P use efficiency in clover has been difficult to achieve (Caradus 1994) and this is also likely to be the case for ryegrass as well. There is also a risk that breeding for improved P utilisation in forages may reduce the availability of P to ruminants, which could increase the occurrence of hypophosphatemia related diseases, particularly in dairy cattle (Grünberg 2008).

Greenhouse gas emissions (CO_2 and N_2O) are a concern for the environment. Internationally, greenhouse gases are largely the result of fossil fuel use, while in New Zealand the low population of people and the high number of ruminants means emissions are largely from livestock, with a smaller component from on-farm energy use.

The fossil fuel energy used to make N (or other) fertilisers is a major sustainability concern. Energy efficiency on many farms using high rates of N fertiliser can be very low when compared to mixed clover-based systems, which are usually much more resource efficient (Basset-Mens, Ledgard and Carran 2005; Andrews *et al*. 2007; Abberton *et al*. 2008) and profitable despite offering a lower animal production to those involving high rates of N (Clark and Harris 1995; Humphreys, Casey and Laidlaw 2009; Woodfield and Clark 2009). It is clear that farms using high rates of N fertiliser developed from fossil fuels are unsustainable in the long term and breeding ryegrass for more efficient N use may help mitigate this slightly, but the "ecological footprint" would be much lower if clovers were to become the major source of N.

Atmospheric CO_2 levels are increasing in an unsustainable way from the burning of fossil fuels, from a mole fraction of 280 ppm in pre-industrial times to 390 ppm in 2010 (+39%), and is currently increasing by an alarming 2 ppm each year. The extra insulating effect on the earth contributes to the observed global warming. Elevated CO_2 concentrations also increase pasture productivity, as experiments show that C3 grasses respond to elevation of CO_2 concentration by producing more biomass (Newton and Edwards 2006). Cultivars vary in their ability to utilise higher CO_2 levels and it is likely that part of the increase in pasture yield achieved through breeding has been due to adaptation to the higher CO_2 levels of today.

Perennial ryegrass pastures in Europe are already harvested for biofuel production, with substantial EU investment in biorefining infrastructure. Ryegrasses bred for large infrequent harvests are required although the quality parameters for biofuel production may be quite different to those demanded for animal grazing or silage production.

The energy harvested from any biofuel crop needs to be much greater than the fossil fuel energy consumed during the manufacture of the N fertiliser and other inputs. Trials of new grasses, bred for this purpose at IBERS, have indicated annual yield and quality sufficient to allow a competitive cost of production. European countries developing or already running grass based biorefineries include: Ireland, Belgium, Austria, Denmark, Poland, Germany, the Netherlands and the UK (Charlton *et al*. 2009).

Farm and trial evaluation systems

Increasing animal production on farms is the ultimate goal, but the difficulty and prohibitive expense of measuring this directly for each new cultivar means that practical indirect evaluation systems are used. These typically involve measuring the DM yield and forage quality in replicated plot trials under a cutting management designed to simulate on-farm systems. Usually this is done at multiple locations around a region or country. In some evaluation systems it is necessary to simulate two (or more) farming systems, for example silage and grazing, as it is known that plant morphology and cutting frequency may interact and influence quality and subsequent animal production (Hazard and Ghesquière 1997; Flores-Lesama *et al*. 2006).

Large-scale animal production trials have shown highly significant differences between cultivars under both grazing and conservation managements. These trials also demonstrated the importance of total yield, seasonal growth and quality in determining seasonal and total annual animal production (Connolly *et al*. 1977; Connolly 1979).

In some countries the management of official trials has now been standardised and regulated, often to such an extent that breeders' trials will mimic the official trials very closely. In these situations breeders are not motivated to select for traits other than those measured in such trials. If a breeder selects to improve additional traits, these must demonstrate clear, marketable added value to farmers. Conversely, other countries lack any regulatory requirement, and even uncertified seed may be sold, sometimes under a cultivar name, to the detriment of the seed and livestock industries.

The question of how best to evaluate cultivars must always be critically examined. The evaluation systems must not only be relevant to on-farm grazing systems, they must also provide clear motivation to breeders to select for the most relevant traits. Furthermore, evaluation systems must be prepared to change to reflect any changing farm management practices (Gilliland *et al*. 2002; Conaghan *et al*. 2008). This can be particularly difficult when farm systems vary in their use of different cultivar types, grazing systems and clover management.

Although ryegrass yield and quality, as measured in cutting trials, are generally strongly associated with animal performance, this may not always be the case. In some situations there are differences in ranking between the results from cutting and grazing (Camlin and Stewart 1975; Orr, Martyn and Clements 2001). Particular disparity may occur between trials and farm use when the cutting system used allows a large herbage mass to develop while on-farm management involves grazing frequently or continuously. Grazing trials are also run in some situations, but they are more complex and expensive. They will require animals and more complex yield assessment, such as pre-trim and post harvest measurements on rotating sub-samples of the plot.

In mixed swards of ryegrass and clovers, or indeed with any herb or weed species, ryegrass is only one component of the pasture ecosystem, and the factors driving animal production are naturally more complex (Lüscher *et al*. 2008). Lifting the harvestable yield and quality of this mixed-sward ecosystem, and hence the animal productivity, is much more difficult than breeding improved ryegrass cultivars for a ryegrass monoculture. For this reason the genetic gains for mixed-sward yield will usually be much less than in comparable single-species situations (Frankow-Lindberg *et al*. 2009). Understanding the interactions that occur in mixed swards is crucial to improving productivity in these systems.

In mixed ryegrass-clover swards it is possible for relatively small fluctuations in the proportion of clover species to disproportionately influence animal production and outweigh small changes in ryegrass yield (Hyslop *et al*. 2000). Yield of the ryegrass component may be a poor predictor of animal performance in such circumstances, particularly when any additional ryegrass yield may suppress the clover. Breeding high yielding ryegrasses that are less aggressive towards clover would be the ideal solution, but despite observations that the more open tetraploid cultivars often support more clover in New Zealand, there are no reports of breeders targeting clover compatibility as a breeding objective.

In situations where animals are offered only a partial pasture diet the nutritional requirements from pasture are likely to be considerably different to those demanded from 100% pasture feeding. In situations where nutrient-rich supplements are fed, the yield of ryegrass is likely to be even more paramount than its quality when compared to a situation where pasture is the sole diet.

The development of an effective farming system seldom relies upon a single ryegrass type over the whole farm, as farmers continually manipulate a broad range of pasture, supplementary crop and feed purchase options to provide a continuous cost-effective seasonal flow of feed to match the animal feed demands. This may involve a range of ryegrass types on different areas of the farm, pure or in legume mixtures, combined with supplementary feed crops such as brassicas or maize, often backed up by more expensive feed purchase options. Systems like this make ryegrass trait prioritisation more difficult than for simpler farm systems.

Trait prioritisation

To place an economic priority on each trait in a breeding programme requires knowledge of their relative value in farm systems. The relative economic value of various factors will usually coincide with the management factors that farmers have identified as important drivers of their production. In many cases the impact of management will be much larger than the magnitude of any genetic advance but small genetic advances can often be magnified by on-farm management. Economic weights have been developed for important production factors in the context of the Irish dairy industry (McEvoy *et al*. 2010). Converting farm economic weightings to ryegrass trait priorities will be easier to determine for farms using pure ryegrass swards than those using clover mixtures or supplementary brassica crops.

Once the economic priority for each trait has been determined the breeder must then balance these against the genetic variation, heritability and likely progress that can be made. Some traits may be particularly difficult to improve through lack of genetic variation, lack of a suitable selection methodology or, indeed, lack of funds. Detailed sustained research and focus may be required on such traits in order to make useful progress (Parsons *et al*. 2010).

In the end though, the breeder has to develop a cultivar with a strong overall balance of traits, none of which can be deficient.

Future breeding opportunities

A wide range of traditional breeding methods are employed by ryegrass breeders, including recurrent selection, mass selection of ecotypes, tetraploidy, hybridisation between elite cultivars, introgression of "new" germplasm, and hybrid production involving male sterility, to name a few. As breeding is a long-term process it is possible to predict, with some accuracy, potential cultivar releases in the next 10 years as breeding programmes have already commenced. Genetic progress over this period is likely to be similar to that in the past, with much slower progress possible in the more complex ecosystems of mixed swards than with pure ryegrass systems.

Fescue introgression to enhance genetic variation

One of the more interesting breeding opportunities for perennial ryegrass improvement is to extend the genetic variation available to ryegrass breeders by introgression of chromosome segments or genes from closely related *Lolium* and *Festuca* species. This added genetic diversity opens prospects of making more progress in breeding for certain traits than is possible within perennial ryegrass alone. Much of this work has been led by Humphreys *et al.* (2006) and colleagues at IBERS in the UK. The similar, but more general, approach of crossing *Lolium* and *Festuca* species to develop Festulolium has been undertaken by many breeders. With the knowledge gained, there is now significant potential to use introgression breeding from closely related *Festuca* species provided funding is maintained.

The role of genetic modification

Genetic modification (GM) is now becoming widespread in many major crops throughout the world with a number of valuable traits available. Indeed it is clear that GM is expanding into more crop species and more regions of the world and involving a greater range of useful traits.

As yet there has not been a release of any genetically modified forage grass, although a number of research projects are underway. These include such targets as improved quality through increased fibre digestibility, reduced lignin, increased condensed tannins, increased fructan accumulation, increased lipid concentration, improved salt tolerance, as well as increased drought tolerance (Smith, Spangenberg and Stewart 2007; Puthigae *et al*. 2010). Field trials have been carried out with ryegrass plants in Europe, USA and Australia (Badenhorst *et al*. 2010).

Improved animal production from ryegrass pastures will require the use of appropriate genes. However, it is difficult to find valuable single or multiple genes due to the complexity of pasture, rumen digestion and animal production systems. One of the barriers to the use of genetically modified perennial ryegrass is likely to be public acceptance. However, this lack of public acceptance seems unjustifiable when GM-based medicines are widely used (and demanded), garments based on GM cotton are widely worn, and many of the existing GM crops, such as maize, soybean and cotton seed, are already imported and used in forage systems internationally, even in some jurisdictions where GM crops are not grown.

Perhaps the greatest barrier to the introduction of GM forage crops is simply economic, in that investment in forages is limited by the scale of the seed industry, and GM regulatory processes pose an enormous economic limitation (Stewart and Woodfield 2009).

In the future, it is likely that research will continue with genetic modification in some jurisdictions although only a few of the current GM traits under investigation are likely to be of value on farms and result in commercial cultivars. Greater investment will be required in this area in order to incorporate genes of known value currently in use in other crops.

Funding of ryegrass breeding

In many parts of the world Government investment in breeding of forage plants is becoming more limited and breeding is being left increasingly to seed companies. The seed industry has a very limited scale when compared to livestock industries. To put this in perspective it only requires 150 m^2 of ryegrass seed crop to maintain the average Irish dairy farm when pasture is renewed at the current rate of 2% annually (O'Donovan *et al*. 2010). The total amount of ryegrass seed used in Ireland annually could be grown on 2000 ha, which represents only a few large seed production farms in Oregon or New Zealand. Although small, it is crucial that the seed industry remains viable as it is not only responsible for delivery of seed but also for funding the majority of ryegrass breeding. This means that investment in breeding will be subject to the economic success of the companies involved and is unlikely to increase. Indeed, continued changes in the seed industry could even leave many ryegrass breeding programmes vulnerable in the future.

Limited funding may also mean that investment is unlikely to be focussed on necessary long-term breeding programmes, or the more expensive DNA-based technologies. These technologies offer breeders a wider range of methods, many enabling very precise knowledge of genetic systems. Many of these methods require expensive research to implement and ryegrass breeders look forward to the day when they can afford to measure large numbers of plants. The best example of these technologies is that of molecular-marker assisted selection but it is fair to say that ryegrass breeders are only beginning to use these tools.

It is also likely that a greater disconnect will emerge between practical field breeding, carried out by seed companies, and the greater concentration of academic laboratory-based high-tech DNA research promoted within state-funded research institutes. Unless viable linkages can be made between seed companies and these research institutes, DNA-based technologies are unlikely to contribute greatly to future cultivar improvement.

References

Abberton, M.T., Marshall, A.H., Humphreys, M.W., Macduff, J.H., Collins, R.P. and Marley, C.L. 2008. Genetic improvement of forage species to reduce the environmental impact of temperate livestock grazing systems. *Advances in Agronomy* **98:** 311–355.

Abberton, M.T. and Marshall, A.H. 2010. White clover. Handbook of plant breeding Volume 5. In: "Fodder Crops and Amenity Grasses", (eds. B. Boller, U.K. Posselt and F. Veronesi). Springer, New York, ISBN:9781441907592, pages 457–476.

Allsop, A.R.C., Nicol, A.M. and Edwards, G.R. 2009. Effect of water soluble carbohydrate concentration of ryegrass on the partial preference of sheep for clover. *Proceedings of the New Zealand Animal Production Society* **69:** 20–23.

Andrews, M., Scholefield, D., Abberton, M.T., McKenzie, B.A., Hodge, S. and Raven, J.A. 2007. Use of white clover as an alternative to nitrogen fertiliser for dairy pastures in nitrate vulnerable zones in the UK: productivity, environmental impact and economic considerations. *Annals of Applied Biology* **151:** 11–23.

Annicchiarico, P. and Proietti, S. 2010. White clover selected for enhanced competitive ability widens the compatibility with grasses and favours the optimization of legume content and forage yield in mown clover-grass mixtures. *Grass and Forage Science* **65:** 318–324.

Armstead, I.P., Turner, L.B., Marshall, A.H., Humphreys, M.O., King, I.P. and Thorogood, D. 2008. Identifying genetic components controlling fertility in the outcrossing grass species perennial ryegrass (*Lolium perenne*) by quantitative trait loci analysis and comparative genetics. *New Phytologist* **178:** 559–571.

Badenhorst, P.E., Dimech, A.M., Panter, S., Liu, Z., Ramage, C., Stewart, A.V., Smith, K.F., Mouradov, A. and Spangenberg, G.C. 2010. Field evaluation of transgenic perennial ryegrass (*Lolium perenne* L.) plants for enhanced fructan accumulation and biomass yield. Molecular Breeding of Forage and Turf Conference, Buenos Aires 15th–19th March 2010.

Basset-Mens, C., Ledgard, S. and Carran, A. 2005. First life cycle assessment of milk production from New Zealand dairy farm systems, AgResearch Ltd. www.anzsee.org/anzsee2005papers/Basset-Mens_LCA_NZ_milk_production.pdf

Brereton, A.J. and McGilloway, D.A. 1999. Winter growth of varieties of perennial ryegrass (*Lolium perenne* L.). *Irish Journal of Agricultural and Food Research* **38:** 1–12.

Bryant, R.H., Parsons, A.J., Rasmussen, S. and Edwards, G.R. 2009. Pasture production and botanical composition of high sugar and control ryegrasses with or without endophyte under irrigation in Canterbury. *Proceedings of the New Zealand Grassland Association* **71:** 177–185.

Camlin, M.S. 1997. Plant breeding – Achievement and prospects – Grasses. In: "Seeds of Progress", (ed. J.R. Weddell). Occasional Symposium No. 31. British Grassland Society, pages 2–14.

Camlin, M.S. and Stewart, R.H. 1975. Reaction of Italian ryegrass cultivars under grazing as compared with cutting. *Grass and Forage Science* **30:** 121–130.

Camlin, M.S. and Stewart, R.H. 1978. The assessment of persistence and its application to the evaluation of mid season and late perennial ryegrass cultivars. *Journal of the British Grassland Society* **33:** 275–282.

Caradus, J.R. 1994. Achievements in improving adaptation of forages to acid, low phosphorus soils. In: "Proceedings of Workshop on Adoption of Plants to Soil Stresses", Lincoln University of Nebraska, pages 295–327.

Charlton, A., Elias, R., Fish, S., Fowler, P. and Gallagher, J.A. 2009. Biorefining opportunities in Wales: understanding the scope for building a sustainable, biorenewable economy using plant biomass. *Chemical Engineering Research and Design* **87:** 1147–1161.

Clark, D.A. and Harris, S.L. 1995. "White clover or nitrogen fertiliser for dairying?" Agronomy Society of New Zealand Special Publication No. 11, pages 107–114.

Conaghan, P., Casler, M.D., McGilloway, D.A., O'Kiely, P. and Dowley, L.J. 2008. Genotype - environment interactions for herbage yield of perennial ryegrass sward plots in Ireland. *Grass and Forage Science* **63:** 107–120.

Connolly, V. 1979. Influence of pasture type on animal production. *Irish Grassland and Animal Production Association Journal* **14:** 5–9.

Connolly, V. 2001. Breeding improved varieties of perennial ryegrass. Teagasc – Project Report – 3495, ISBN 1 84170 199 8, 17 pages.

Connolly, V., Ribeiro, M.A.M. and Crowley, J.G. 1977. Potential of grass and legume cultivars under Irish conditions. In: "Proceedings of the International Meeting on Animal Production from Temperate Grassland", (ed. B. Gilsenan). Dublin, pages 379–387.

Cosgrove, G.P., Koolaard, J., Potter, F., Luo, D., Burke, J.L. and Pacheco, D. 2010. Milksolids yield from high water soluble carbohydrate ryegrasses: a combined analysis of multiyear data. In: "Proceedings of the 4th Australasian Dairy Science Symposium", 31st August–2nd September, Lincoln University, New Zealand, pages 334–338.

Crush, J.R. and Nichols, S.N. 2010. Progress towards forage plant systems for sustainable dairying. In: "Proceedings of the 4th Australasian Dairy Science Symposium", 31st August–2nd September, Lincoln University, New Zealand, pages 50–52.

Easton, H.S. 2007. Grasses and *Neotyphodium* endophytes: co-adaptation and adaptive breeding. *Euphytica* **154:** 95–306.

Easton, H.S., Amyes, J.M., Cameron, N.E., Green, R.B., Kerr, G.A., Norriss, M.G. and Stewart, A.V. 2002. Pasture plant breeding in New Zealand: Where to from here?. *Proceedings of the New Zealand Grassland Association* **64:** 173–179.

Easton, H.S., Stewart, A.V., Lyons, T.B., Parris, M. and Charrier, S. 2009. Soluble carbohydrate content of ryegrass cultivars. *Proceedings of the New Zealand Grassland Association* **71:** 59–64.

Edwards, G.R., Parsons, A.J. and Rasmussen, S. 2007. High sugar grasses for dairy systems. In: "Meeting the Challenges for Pasture Based Dairying". Proceedings of the 3rd Dairy Science Symposium Melbourne, Australia, pages 307–334.

Elgersma, A. 1990. Seed yield related to crop development and to yield components in nine cultivars of perennial ryegrass (*Lolium perenne* L.). *Euphytica* **49:** 141–154.

Evans, D.R., Williams, T.A. and Evans, S.A. 1996. Breeding and evaluation of new white clover varieties for persistency and higher yields under grazing. *Grass and Forage Science* **51:** 403–411.

Flores-Lesama, M., Hazard, L., Betin, M. and Emile, J. 2006. Differences in sward structure of ryegrass cultivars and impact on milk production of grazing dairy cows. *Animal Research* **55:** 25–36.

Francis, S.A., Chapman, D.F., Doyle, P.T. and Leury, B.J. 2006. Dietary preferences of cows offered choices between white clover and "high sugar" and "typical" perennial ryegrass cultivars. *Australian Journal of Experimental Agriculture* **46:** 1579–1587.

Frankow-Lindberg, B.E., Brophy, C., Collins, R.P. and Connolly, J. 2009. Biodiversity effects on yield and unsown species invasion in a temperate forage ecosystem. *Annals of Botany* **103:** 913–921.

Fulkerson, W.J. and Donaghy, D.J. 2001. Plant soluble carbohydrate reserves and senescence – key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Australian Journal of Experimental Agriculture* **41:** 261–275.

Gilliland, T.J. and Mann, R.L. 2001. The silage yield potential of hybrid ryegrass over a 5 year period in Northern Ireland relative to other ryegrasses and timothy. *Grass and Forage Science* **56:** 305–310.

Gilliland, T.J., Barrett, P.D., Mann, R.L., Agnew, R.E. and Fearon, A.M. 2002. Canopy morphology and nutritional quality traits as potential grazing value indicators for *Lolium perenne* varieties. *Journal of Agricultural Science* **139:** 257–273.

Grünberg, W. 2008. Phosphorus homeostasis in dairy cattle: some answers, more questions. In: "Proceedings of the 17th Annual Tri-State Dairy Nutrition Conference", Fort Wayne, Indiana, USA, 22–23 April, pages 29–35.

Hageman, I.W., Lantinga, E.A., Schlepers, H. and Neyteboom, J.H. 1993. Herbage intake, digestibility characteristics and milk production of a diploid and two tetraploid cultivars of perennial ryegrass. In: "Proceedings of the XVII International Grassland Congress", pages 459–460.

Hazard, L. and Ghesquière, M. 1997. Productivity under contrasting cutting regimes of perennial ryegrass selected for short and long leaves: *Euphytica* **95:** 295–299.

Hume, D.E., Cooper, B.M. and Panckhurst, K.A. 2009. The role of endophyte in determining the persistence and productivity of ryegrass, tall fescue and meadow fescue in Northland. *Proceedings of the New Zealand Grassland Association* **71:** 121–126.

Humphreys, M.W., Yadav, R.S., Cairns, A.J., Turner, L.B., Humphreys, J. and Skot, L. 2006. A changing climate for grassland research. *New Phytologist* **169:** 9–26.

Humphreys, J., Casey, I.A. and Laidlaw, A.S. 2009. Comparison of milk production from clover-based and fertilizer-N-based grassland on a clay-loam soil under moist temperate climatic conditions. *Irish Journal of Agricultural and Food Research* **48:** 189–207.

Hyslop, M.G., Fraser, T.J., Smith, D.R., Knight, T.L., Slay, M.W.A. and Moffat, C.A. 2000. Liveweight gain of young sheep grazing tall fescue or perennial ryegrass swards of different white clover content. *Proceedings of the New Zealand Society of Animal Production* **60:** 51–5.

Latch, G.C.M. 1977. Incidence of barley yellow dwarf virus in ryegrass pastures in New Zealand. *New Zealand Journal of Agricultural Research* **20:** 87–89.

Longland, A.C. and Byrd, B.M. 2006. Pasture non-structural carbohydrates and equine laminitis. *Journal of Nutrition* **136:** 2099S–2102S.

Lüscher, A., Finn, J.A., Connolly, J., Sebastia, M.T., Collins, R.P., Fothergill, M., Porqueddu, C., Brophy, C., Huguenin-Elie, O., Kirwan, L., Nyfeler, D. and Helgadottir, A. 2008. Benefits of sward diversity for agricultural grasslands. *Biodiversity* **9:** 29–32.

Marley, C.L., Fraser, M.D., Fisher, W.J, Forbes, A.B., Ones, R., Moorby, J.M., MacRae, J.C. and Theodorou, M.K. 2007. Effects of continuous or rotational grazing of two perennial ryegrass varieties on the chemical composition of the herbage and the performance of finishing lambs. *Grass and Forage Science* **62:** 255–264.

McEvoy, M., O'Donovan, M. and Shalloo, L. 2010. Evaluating the economic performance of grass varieties. *Advances in Animal Biosciences* **1:** 328.

Merry, R.J., Lee, M.R.F., Davies, D.R., Dewhurst, R.J., Moorby, J.M., Scollan, N.D. and Theodorou, M.K. 2006. Effects of high-sugar ryegrass silage and mixtures with red clover silage on ruminant digestion. 1. *In vitro* and *in vivo* studies of nitrogen utilization. *Journal of Animal Science* **84:** 3049–3060.

Minobe, S., Kuwano-Yoshida, A., Komori, N., Xie, S.P. and Small, R.J. 2008. Influence of the Gulf Stream on the troposphere. *Nature* **452:** 206–209.

Morgavi, D.P., Forano, E., Martin, C. and Newbold, C.J. 2010. Microbial ecosystem and methanogenesis in ruminants. *Animal* **4:** 1024–1036.

Newton, P.C.D. and Edwards, G.R. 2006. Plant breeding for a changing environment. In: "Agroecosystems in a Changing Climate" (eds. P.C.D. Newton, R.A. Carran, G.R. Edwards and P.A. Niklaus), CRC Press, Boston, MA, USA, pages 309–319.

O'Donovan, M. and Delaby, L. 2005. A comparison of perennial ryegrass cultivars differing in heading date and grass ploidy with spring calving dairy cows grazed at two different stocking rates. *Animal Research* **54:** 337–350.

O'Donovan, M., Lewis, E. and O'Kiely, P. 2011. Requirements of future grass based ruminant production systems in Ireland. *Irish Journal of Agricultural and Food Research* **50:** 1–21.

Orr, R.J., Martyn, M. and Clements, R.O. 2001. Evaluation of perennial ryegrass varieties under frequent cutting or continuous stocking with sheep. *Plant Varieties and Seeds* **14:** 181–199.

Orr, R.J., Cook, J.E., Young, K.L., Champion, R.A. and Rutter, S.M. 2005. Intake characteristics of perennial ryegrass varieties when grazed by yearling beef cattle under rotational grazing management. *Grass and Forage Science* **60:** 157–167.

Parsons, A.J., Edwards, G.R., Newton, P.C.D., Chapman, D.F., Caradus, J.R., Rasmussen, S. and

Rowarth, J.S. 2010. Past lessons and future prospects: plant breeding for cool temperate pastures. In: "Proceedings of the 4th Australasian Dairy Science Symposium", 31st August–2nd September, Lincoln University, New Zealand, pages 272–291.

Pollock, C.J. and Cairns, A.J. 1991. Fructan metabolism in grasses and cereals. *Annual Review of Plant Physiology and Plant Molecular Biology* **42:** 77–101.

Popay, A.J. and Thom, E.R. 2009. Endophyte effects on major insect pests in Waikato dairy pasture. *Proceedings of the New Zealand Grassland Association* **71:** 121–126.

Puthigae, S., Templeton, K., Sontam, D., Whittaker, D., Bryant, C., Jones, C., Gill, G., Richardson, K. and Hanley, Z. 2010. Engineering drought tolerance in Perennial Ryegrass (*Lolium perenne* L.) using its own genome. In: "Plant Biology 2010", American Society of Plant Biologists, Poster 07001.

Rasmussen, S., Parsons, A.J., Fraser, K., Xue, H. and Newman, J.A. 2008. Metabolic profiles of *Lolium perenne* are differentially affected by nitrogen supply, carbohydrate content, and fungal endophyte infection. *Plant Physiology* **146:** 1440–1453.

Rasmussen, S., Parsons, A.J., Xue, H. and Newman, J.A. 2009. High sugar grasses – harnessing the benefits of new cultivars through growth management. *Proceedings of the New Zealand Grassland Association* **71:** 167–175.

Rennie, G.M., King, W.M. and Bell, N.J. 2010. Delivering endophyte benefits on-farm: pasture renewal success and effects on pasture pests. In: "Proceedings of The International Symposium on Fungal Endophytes of Grasses", (CD-ROM), June 28–July 1, Kentucky, USA.

Ribeiro, M.A.M., Do Valle, Gurney, K.A. and Bush, L.P. 1996. Endophyte *Acremonium lolii* in ecotypes of perennial ryegrass (*Lolium perenne* L.) collected in old Irish pastures. *Irish Journal of Agricultural and Food Research* **35:** 151–157.

Rolston, M.P., Chynoweth, R.J. and Stewart, A.V. 2006. Forage seed production: 75 years applying science and technology. *Proceedings of the New Zealand Grassland Association* **68:** 15–24.

Rolston, P., Trethewey, J., McCloy, B. and Chynoweth, R. 2007. Achieving forage ryegrass seed yields of 3000 kg/ha and limitations to higher yields. In: "6th International Herbage Seed Conference", Norway, pages 100–106.

Schejbel, B., Jensen, L.B., Xing, Y. and Lübberstedt, T. 2007. QTL analysis of crown rust resistance in perennial ryegrass under conditions of natural and artificial infection. *Plant Breeding* **126:** 347–352.

Seager, R., Battisti, D.S., Yin, J., Gordon, N., Naik, N., Clement, A.C. and Cane, M.A. 2002. Is the Gulf Stream responsible for Europe's mild winters? *Quarterly Journal of the Royal Meteorological Society* **128:** 2563–2586.

Smith, G. 2008. How endophyte and breeding for high sugar grasses affects allocation patterns in perennial ryegrass. Lincoln University, BAgrSc, Honours Dissertation.

Smith, K.F., Spangenberg, G. and Stewart, A. 2007. Breeding high energy grasses: how, why and what are the benefits to producers. *Proceedings of the New Zealand Grassland Association* **69:** 173–177.

Sprosen, M.S., Ledgard, S.F. and Thom, E.R. 1997. Nitrate leaching is similar in N2-fixing grass-clover pasture and N-fertilised grass-only pasture at similar N inputs. *Proceedings of the New Zealand Grassland Association* **59:** 125–128.

Stewart, A.V. 2001. A comparison of nitrogen leaching from plots of perennial ryegrass (*Lolium perenne* L.) and winter active Swamp Phalaris (*Phalaris arundinacea* subsp. *oehleri* Pilger) In: "New Zealand Land Treatment Collective Conferences", Invercargill, NZ, pages 109–115.

Stewart, A.V. 2006. Genetic origins of New Zealand perennial ryegrass (*Lolium perenne*) cultivars. In: "Proceedings of the 13th Australasian Plant Breeding Conference", Christchurch, New Zealand, pages 11–20.

Stewart, A.V. and Woodfield, D. 2009. A conventional breeders view of GM. *AgScience* **36:** 11–13.

Stewart, A.V., Easton, H.S., Lyons, T.B., Parris, M. and Charrier, S. 2009. Improving soluble carbohydrates content of perennial ryegrass herbage. *Journal of Breeding and Genetics* Vol. 41, Special Supplement, August 2009. ISSN 102907073 (CD-ROM).

Thom, E.R., Waugh, C.D. and Minneé, E.M.K. 2010. Dairy cow responses to the wild-type endophyte in perennial ryegrass. In: "Proceedings of the 4th Australasian Dairy Science Symposium", 31st August–2nd September, Lincoln University, New Zealand, pages 370–375.

Thomson, N. and Kay, J. 2005. Milksolids production from cows grazing timothy/white clover in comparison with ryegrass pastures. *Proceedings of the New Zealand Grassland Association* **67:** 123–130.

Thorogood, D., Paget, M.F., Humphreys, M.O., Turner, L.B., Armstead, I.P. and Roderick, H.W. 2001. QTL analysis of crown rust resistance in perennial ryegrass – implications for breeding. *International Turfgrass Society Research Journal* **9:** 218–223.

van Bogaert, G. 1975. A comparison between colchicine induced tetraploid and diploid cultivars of *Lolium* species. In: "Ploidy in Fodder Plants". *Report of the Eucarpia Fodder Crops Section* (ed. B. Nuesch), Zurich, Switzerland, pages 61–78.

van der Heijden, S.A.G. and Roulund, N. 2010. Genetic gain in agronomic value of forage crops and turf: A review. In: "Sustainable use of Genetic Diversity in Forage and Turf Breeding", (ed. C. Huyghe). Springer, London, UK, pages 247–260.

Vipond, J.E., Swift, G. and McClelland, T.H. 1993. Sustainable clover-dominant swards for lamb production under low temperatures. In: "Proceedings of the XVII International Grassland Congress", pages 674–675.

Webster, D.E., Forster, R.L.S., Sinclair, L. and Guy, P.L. 1996. Distribution of ryegrass mosaic virus in New Zealand perennial ryegrass pastures as determined by ELISA and RT-PCR. *New Zealand Journal of Agricultural Research* **39:** 405–412.

Wilkins, P.W. 1974. Tolerance to ryegrass mosaic virus and its inheritance *Annals of Applied Biology* **78:** 187–192.

Wilkins, P.W. 1998. "AberDart (Ba11778)", IBERS Internal Reference 1830 4468. Institute of Biological, Environmental and Rural Sciences, University of Aberystwyth, United Kingdom.

Wilkins, P.W. and Catherall, P.L. 1977. Variation in reaction to barley yellow dwarf virus in ryegrass and its inheritance. *Annals of Applied Biology* **85:** 257–263.

Wilkins, P.W. and Humphreys, M.O. 2003. Progress in breeding perennial forage grasses for temperate agriculture. *Journal of Agricultural Science* **140:** 129–150.

Wilkins, P.W. and Lovatt, J.A. 2007. "AberMagic (Ba13582)", IBERS Internal Reference 1340 4213. Institute of Biological, Environmental and Rural Sciences, University of Aberystwyth, United Kingdom

Wilkins, P.W., Allen, D.K. and Mytton, L.R. 2000. Differences in the nitrogen use efficiency of perennial ryegrass varieties under simulated rotational grazing and their effects on nitrogen recovery and herbage nitrogen content. *Grass and Forage Science* **55:** 69–76.

Wilkins, P.W., Lovatt, J.A., Hayes, R.C. and Thomas, G.L. 2010. "AberGreen (Ba13926)", IBERS Internal Reference. Institute of Biological, Environmental and Rural Sciences, University of Aberystwyth, United Kingdom

Woodfield, D.R. 1999. Genetic improvements in New Zealand forage cultivars. *Proceedings of the New Zealand Grassland Association* **61:** 3–7.

Woodfield, D.R. and Easton, H.S. 2004. Advances in pasture plant breeding for animal productivity and health. *New Zealand Veterinary Journal* **52:** 300–310.

Woodfield, D.R. and Clark, D.A. 2009. Do forage legumes have a role in modern dairy farming systems? *Irish Journal of Agricultural and Food Research* **48:** 137–147.

Woollings, T. 2010. Dynamical influences on European climate: an uncertain future. *Philosophical Transactions of the Royal Society A* **368:** 3733–3756.